

The spatial resolution and non-contact working conditions of a scanning ion conductance microscope (SICM) are largely determined by the size and shape of the scanning probe tip. Estimating the tip geometry has traditionally required the use of scanning electron microscopy (SEM), a difficult, time-consuming process that, even if successful, provides little information about the crucial inner geometry of the probe. As a result, such measurements are not routinely made. Instead, tip sizes are often crudely estimated from pipette resistances. We have developed a simple method of more precisely estimating the geometry (tip radius and inner cone angle) from multiple resistance measurements recorded during quasi-controlled breakage of the tip. Such measurements can be easily obtained using only the standard SICM apparatus. Results compare favourably with SEM estimates, are more informative and avoid some of the assumptions necessary for SEM estimation.

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Development of Carbon Nano-Heater: Stimulation of Sensory Neurons for Functional Study of Heat Sensitive Channels

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Suspended Bilayers in Shaped Apertures Enable 24-Hour Ion Channel Recordings

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Electrophysiological characterization of reconstituted ion channels requires a stable and electrically addressable membrane, usually an aperture-suspended lipid bilayer in a septum that separates two aqueous compartments having polarizable electrodes for detection of picoampere channel gating events. Unfortunately, bilayers formed in conventional cylindrical apertures in septum materials such as Teflon and polystyrene are relatively fragile. Apertures with a low thickness/diameter ratio are expected to facilitate the formation of more stable bilayers, but thin septa are difficult to handle and also increase electrical noise in the ion channel current measurements due to an increased background capacitance. In this study we used 3D UV-lithography to create tapered apertures in layers of UV-curable polymers, which have similar electrical properties as Teflon or polystyrene sheets. In these beak- or triangle-shaped apertures, the septum thickness at the edge of the aperture, where the bilayer is formed, is substantially smaller than the bulk septum thickness. We established that >50 μm thick layers are sufficiently robust, following release from the substrate, to be used as septum sheets and also exhibit a low background capacitance. The moderate hydrophobicity of these photoresists was mitigated by vapor deposition of Parylene C, resulting in a contact angle of 110°, thus combining the favourable mechanical and electrical properties of traditional septum materials with lithography-enabled control of aperture diameter and shape. Bilayer formation with both the painting and the Montal-Mueller method was demonstrated for shaped apertures with an inner diameter of 60 μm . Significantly, these bilayers were stable for 24 hours or more at potentials >125 mV, and sustained continuous ion channel activity. This remarkable stability is suitable for automated bilayer array formation for high-throughput ion channel measurements.

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Voltage-Gating of Ions and Water in Hydrophobic Nanopores

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The behavior of water in nanopores is very different from bulk water and strongly depends on the chemical characteristics of the pore walls. If the pore walls are lined with hydrophobic groups the density of water was predicted to be significantly lower than in the bulk and if a pore is sufficiently narrow, the water can even evaporate stopping any transport through the membrane. We provided an experimental evidence for the nano-induced water evaporation and condensation by chemically modifying polymer nanopores with decylamines. Hydrophobic properties of the pore walls were confirmed by contact angle measurements of a polymer film subjected to the same modification as the nanopores. The hydrophobic pores are closed for water and ionic transport unless a sufficiently high transmembrane potential is applied. The off state of the pore corresponds to the pore filled with water vapor. The pores conduct ion current only if the water undergoes condensation. The switching between open and closed was found fully reversible over many up

to 20 cycles. For the intermediate voltages the pore would fluctuate between two conducting states. We also found that hydrophobic interactions tune rectifying properties of the pores. Understanding hydrophobic properties in nano-confinement is important in the light of recent findings of the role of hydrophobic interactions in the function of voltage-gated channels. Application of hydrophobic-gating in building drug-delivery systems was investigated with multi-pore membranes.

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A Low Noise Ion Channel Amplifier in a USB Pen Drive

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Ion channels are transmembrane proteins responsible of ion exchange between intra- and extra-cellular environments. Ion channel play an essential role in many biological processes and their malfunctions are involved in several diseases or severe pathologies. As a result, ion channels are targets of numerous pharmaceutical compounds.

The capability of ion channels to respond to different chemical-physical stimuli has inspired the design of hybrid sensors, where they are adopted as sensing units. This approach has already been employed for the detection of molecules in solution and for DNA sequencing.

Both for biology and electronics extents, it is crucial to characterize ion channel electrical behavior with high accuracy and precision. This is achieved using low-noise acquisition equipments able to amplify ion channel picoampere-range currents.

Commercially amplifiers for ion channel recording are bulky instrumentations and are limited in the number of channel simultaneously acquired due to their discrete-architecture electronic limitations. To overcome this weaknesses a single-microchip (integrated circuitry-based) amplifiers have been presented. Unfortunately they suffer from shortcomings like system integration, data elaboration and digital interface, resulting in unsettled systems.

We developed an innovative low-noise technology for ion channel acquisition microchips that allow us to shrink in few squared millimeters the acquisition system improving the performances usually obtained with bulky instruments.

We present our new technology integrated into a microchip and embedded into a USB pen ready to use. The fully comprehensive system includes the amplifier, the data elaboration, the digital interface and the data link.

Furthermore, our technology allows us to configure the system for single or multi-channel acquisition, addressing the needs of emerging microfluidic techniques. Moreover, it offers an extremely flexible input interface and a user programmable stimulus, allowing different setups for different low-noise acquisition needs in different applications.

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Voltage-Induced Pore-Formation and Rupture of Synthetic Lipid Membranes: A High-Throughput Quantitative Voltage Clamp Study on a Lipid Bilayer Array

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Quantitative studies on the electroporation and voltage-induced rupture of synthetic lipid membranes require many experiments under standardized conditions. We used an array platform which allows quick, simultaneous and repetitive membrane formation on 16 identical apertures. Effective voltages for 50% membrane rupture (EV50) were determined for voltage pulse durations of 1.5, 10, 20, 50 and 100ms, different ionic strengths (150, 500 and 3 M KCl), pH (7 or 9) and with and without Ca^{2+} (10 mM), with $n > 300$ per datapoint. EV50 values ranged from 350-550 mV showing subtle but clear changes with high ionic strength, Ca^{2+} (ca. -40 mV) and alkaline pH (ca. +35 mV). To determine the relationship between transient (metastable) pore-formation and irreversible membrane rupture, single bilayers were studied using a high-resolution amplifier (Axopatch200B). In the case of Ca^{2+} , we noted that a lowering of EV50 was accompanied by a decrease, not an increase, in the incidence of pre-rupture pore formation: with 150 mM KCl alone, rupture with detectable prior pore formation occurred in 33% of cases ($n=242$), while, with 10 mM Ca^{2+} added, only 17% of membrane ruptures ($n=53$) were preceded by pore formation.

